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(54) Title: ANTIMICROBIAL SOLUTIONS

(57) Abstract: The invention concerns antimicrobially effective aqueous solutions comprising at least 0.005 % wt of one or more nonionic surfactants which have pH of 9 or above and which do not contain other known antimicrobially active components. The nonionic surfactants are preferably ethoxylated aliphatic alcohols, ethoxylated alkylphenols or amine oxides. The solutions are mild to surfaces and particularly effective against gram-negative bacteria. The invention also concerns a process for antimicrobial treatment of animate or inanimate surfaces using the solutions according to the invention.

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## ANTIMICROBIAL SOLUTIONS

5 Technical field

The invention relates to antimicrobial solutions comprising a nonionic surfactant at alkaline pH.

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Background of the invention

Various cleaning compositions with antimicrobial properties have been described in the art. Such  
15 compositions generally consist of a surfactant system comprising anionic and/or nonionic surfactants and one or more compounds which are responsible for the antimicrobial activity. Particular examples in which a nonionic surfactant is combined with a known antimicrobially active  
20 component to give an antimicrobially active composition have been described e.g. in the patent applications below: EP-A-0766729 describes compositions comprising ethoxylated alcohols in combination with ortho-hydroxybenzoic acid derivatives such as salicylic acid at pH 3-5  
25 EP-A-0874887 describes compositions comprising ethoxylated alcohols and lower alkanols such as ethanol and isopropanol at pH below 6 or above 8.  
US 4,414,128 describes compositions containing 12% betaine or amine oxide and 3% isopropanol at pH 5.5.  
30 EP-A-0478445 describes compositions for disinfection of surgical instruments comprising 13% didecyl-dimethyl ammonium chloride as a biocide and optionally 7% C13/8EO ethoxylated alcohol and 12% ethanol or isopropanol.  
EP-A-0536820 describes acidic compositions containing  
35 nonionic surfactants with HLB 7-10 and 11 and

disinfectants. The compositions may optionally contain up to 2% alcohol to further improve the antimicrobial activity.

5 EP-A-0912678 describes compositions comprising ethoxylated alcohols and/or amine oxides in combination with aromatic alcohols (e.g. benzyl alcohol) or substituted phenols (e.g. thymol, carvacrol and eugenol). The compositions are said to be most effective outside the physiological pH range.

10 Although the prior art mentioned above relates to the use of nonionic surfactants in combination with a large variety of known antimicrobially active compounds, it does nowhere mention the nonionic surfactants to have any antimicrobial activity per se.

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#### Brief Description of the invention

It has now been found that aqueous solutions of nonionic  
20 surfactants per se can be used at alkaline pH to disinfect various surfaces. Such solutions are mild to these surfaces and can therefore often be left on without the need for subsequent rinsing thereby giving a lasting disinfecting action. The solutions are particularly  
25 effective against gram-negative bacteria. The addition to such solutions of other components with known antimicrobial activity is generally not necessary, at least not in a concentration at which they are known to be antimicrobially effective.

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#### Detailed description of the invention

The invention therefore provides antimicrobially effective  
35 aqueous solutions which comprise at least 0.005%wt of one

or more nonionic surfactants, which have  $\text{pH} \geq 9$ , in which the sole antimicrobially active component is the one or more nonionic surfactant(s) and in which the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is antimicrobially effective.

The invention also provides a process for antimicrobial treatment of animate and inanimate surfaces by applying to the surfaces an aqueous solution which comprises at least 0.005%wt of one or more nonionic surfactants, which has  $\text{pH} \geq 9$ , in which the sole antimicrobially active component is the one or more nonionic surfactant(s) and in which the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is antimicrobially effective.

Furthermore the invention provides the use of an aqueous solution which comprises at least 0.005%wt of one or more nonionic surfactants, which has  $\text{pH} \geq 9$ , in which the sole antimicrobially active component is the one or more nonionic surfactant(s) and in which the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is antimicrobially effective, for antimicrobial treatment of animate and inanimate surfaces.

For the purposes of this invention a compound is defined as antimicrobially effective at the concentration at which it is able to give a log reduction of 3 or more in bacterial count in the test described hereinafter in Example 1, using E. coli ATCC 10536 as the test organism and a contact time of at least 5 minutes.

The nonionic surfactants are preferably chosen from:

- ethoxylated aliphatic alcohols  $RO(C_2H_4O)_xH$  wherein R is an aliphatic group containing 6-20 carbon atoms and x is 2-20;
- ethoxylated alkyl-phenols  $R-C_6H_5O(C_2H_4O)_xH$  wherein R is an aliphatic group containing 4-15 carbon atoms and x is 2-20;
- amine oxides  $RRRN^+O^-$  wherein one of R is an aliphatic group containing 6-20 carbon atoms and the other Rs are aliphatic groups containing 1-3 carbon atoms.

More preferably the nonionic surfactant has an HLB of between 9 and 14.

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Particularly suitable nonionic surfactants are the ethoxylated alcohols and alkyl-phenols, more particularly the ethoxylated alcohols. Of the latter compounds, the aliphatic group preferably has 8-18 carbon atoms, more preferably 10-14. The number of ethylenoxy groups is preferably at most 15, more preferably at most 12, whereas the minimum number is preferably at least 3.

The pH of the solutions is preferably at least 9.5, more preferably at least 10, most preferably at least 10.5.

Although antimicrobial effectiveness is maintained at pH 14 or above, solutions of pH above 14 may be excessively aggressive to the surface to be treated and therefore the pH is preferably kept at pH 14 or below. For more

sensitive surfaces such as animal skin or hides the pH is preferably kept at or below 12.5, most preferably below 12. At such pH the solutions are not aggressive to most surfaces and it is therefore often not necessary to rinse

the surface after treatment. Thus, with such solutions even a prolonged antimicrobial effect can be obtained. The desired pH may be given to the solutions of the invention using alkalising agents generally known in the art such as alkali metal hydroxides, alkali metal carbonates, bicarbonates, silicates and phosphates, ammonia or its carbonate or bicarbonate salt, organic amines such as alkanol-amines and combinations thereof. Carbonates and bicarbonates, if necessary in combination with alkali metal hydroxydes, are preferred alkalising agents.

Preferred solutions according to the invention are those which are able to give a log reduction in bacterial count of 3 or more. Concentrations of 0.02%wt of the nonionic surfactant are preferred. More preferably the concentration of nonionic should be 0.05%wt or above. Concentrations of more than 30%wt are in most cases not useful from a practical point of view. Preferably the concentration is below 15%wt.

The solutions according to the invention are effective against a wide range of microorganisms such as gram-positive and gram-negative bacteria and yeasts. They are particularly effective against gram-negative bacterial such as *Escherichia coli* and other coliforms. This makes the solutions particularly useful for disinfecting areas where faecal pollution is a problem.

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#### Minors and Optional Components

Apart from their disinfection properties the solutions according to the invention will generally also have cleaning properties on account of their content of

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nonionic surfactant. The solutions can also contain other minor, unessential ingredients, which aid in their cleaning performance and maintain the stability of the product.

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Thus, the solutions may contain detergent builders. In general, the builder, when employed, preferably will form from 0.1 to 25% by weight of the solution. Sodium carbonate, sodium bicarbonate and mixtures thereof are

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suitable builders.

Optionally, the solution can include one or more amphoteric surfactants, preferably betaines, or other surfactants such as alkyl-amino-glycinates. Betaines may

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be useful for reasons of cost, low toxicity and wide availability.

Typically, betaines in solutions according to the invention are the amido-alkyl betaines, particularly the

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amido-propyl betaines, preferably having an aliphatic alkyl radical of from 8 to 18 carbon atoms and preferably having a straight chain and other betaines, such as alkyl betaines.

Typical levels of amphoteric range from 0.01 to 35%, with levels of 1-20wt% being preferred.

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Anionic surfactants may only be present in minor amounts. If present, they should amount to no more than 30%wt of the total amount of surfactant in the solutions, preferably no more than 10%. For most applications solutions without any anionic surfactant are preferred.

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Metal ion sequestrants, including ethylene diamine-tetra-acetates, amino-polyphosphonates (such as those in the DEQUEST<sup>®</sup> range) and phosphates and a wide variety of other poly-functional organic acids and salts, can also be

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employed. The hygiene performance of the solution may be improved by the presence of a metal ion sequestrant. Suitable hydrotropes include, alkali metal toluene sulphonates, urea, alkali metal xylene and cumene  
5 sulphonates, polyglycols, >20EO ethoxylated alcohols and glycols. Preferred amongst these hydrotropes are the sulphonates, particularly the cumene, xylene and toluene sulphonates. Typical levels of additional hydrotrope range from 0-5% for the sulphonates. The cumene sulphonate is  
10 the most preferred hydrotrope.

Optionally the solutions according to the invention can also contain, in addition to the ingredients already mentioned:

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- various other ingredients to improve the cleaning performance such as solvents, soil suspending agents and  
detergent enzymes;
- various ingredients to improve physical stability such  
20 as gel-control agents and freeze-thaw stabilisers
- various ingredients to improve consumer appeal such as colorants and perfumes.

For many purposes the presence of any other surfactant  
25 than the nonionics disclosed above or any other ingredient to improve the cleaning performance is unnecessary or may be even undesirable. More particularly the invention expressly also provides aqueous solutions consisting only of nonionic surfactant in the concentrations indicated  
30 above, an alkalising agent to give the desired pH and, if desired, ingredients to improve stability and/or consumer appeal.

### 35 Example 1



The antimicrobial efficacy of Neodol 91-8E<sup>TM</sup> was tested using *Escherichia coli* ATCC 10536 as the test microorganism. Neodol 91-8E is a mixture of C9-C11 aliphatic alcohols ethoxylated with (average) 8 ethyleneoxy groups and is obtainable from Shell.

#### Bacterial Inoculum Preparation

A bead was removed from the storage vial and aseptically transferred to 100ml Tryptone Soya Broth in a 250ml capacity conical flask stoppered with a sponge bung. The broth was incubated at 37°C in an orbital shaker or shaking waterbath for 24 hours. After incubation, the culture suspension was aseptically transferred into two 50ml centrifuge tubes and centrifuged at 2180g for 10 minutes to harvest the cells. The supernatant was decanted and pellets resuspended in peptone diluent (0.1 percent peptone and 0.85 percent sodium chloride, pH 7.0). The suspensions were kept on ice until needed, and then the bacterial suspensions were further diluted 1/100 in peptone diluent (giving approximately  $1 \times 10^8$  cells per ml), and left on the bench at room temperature for at least 30 minutes before use in the disinfectancy test.

#### 50mM carbonate/bicarbonate buffer:

Solution A: 14.23g/l sodium carbonate decahydrate

Solution B: 4.2g/l sodium hydrogen carbonate

800mls solution A added to 200mls solution B, pH adjusted to 10.5 with 1M HCl

#### Formulation Preparation

A solution of 5 percent Neodol 91-8E was prepared in 50mM pH 10.5 carbonate/bicarbonate buffer. A series of dilutions was

carried out in pH 10.5 buffer (1/10, 1/20, 1/40, 1/80 and 1/160.

### Biocidal Efficacy Test

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The test used was a microtitre plate assay, which is comparable to 'Method for the test for the antimicrobial activity of disinfectants in food hygiene', more commonly known as the European Suspension Test (EST). Water of  
10 Standard Hardness and bovine albumin were not included in these tests.

The microtitre plate (Bibby Sterilin, sterile 96-well, flat-bottom) were prepared by adding quench solution (270µl) in  
15 row B and peptone diluent (270µl) into rows C-G. The quench solution was composed of Tween 80 (3.0 percent), lecithin (0.3 percent), L-histidine (0.1 percent), sodium thiosulphate (0.5 percent) and potassium dihydrogen phosphate buffer (0.25N, 1 percent) in sterile distilled water (1 litre).  
20

Two aliquots (270µl) of each dilution of the formulation was added to two wells of row A of a microtitre plate. An aliquot of microbial suspension (30µl) was then added simultaneously to all the wells of row A of the microtitre plate using a  
25 multichannel pipette. The test mixture was left at 20°C for a contact time of 5 minutes ± 5 seconds, after which time an aliquot (30µl) was transferred into row B (quench), giving a  $10^{-1}$  dilution. After 5 ± 1 min, an aliquot (30µl) was transferred to row C (containing peptone diluent, maximum  
30 recovery medium). Further serial dilutions were performed in the same manner until row G ( $10^{-6}$  dilution). The bacteria were enumerated using the Miles-Misra technique. Three aliquots (10µl) from each dilution were spotted onto pre-

dried Tryptone Soya Agar plates which had been divided into 6 sectors. The spots were allowed to dry and the plates incubated at 37L for 24 hours. Plates were counted by selecting a dilution segment with 3-50 cfu per spot and the average of the 3 spots was calculated. The decadic logarithm of this number was calculated and subtracted from the decadic logarithm of the initial count to give the log (reduction). Plates without colonies were recorded as 'no growth'.

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Results

Formulation Dilution	Initial Inoculum in Test (30µl)	Log (Reduction)	
		rep 1	rep 2
1/10	5.8	>4.8	>4.8
1/20	5.8	>4.8	>4.8
1/40	5.8	>4.8	>4.8
1/80	5.8	>4.8	4.3
1/160	5.8	2.6	2.6

CLAIMS

1. Antimicrobially effective aqueous solutions characterised in that they:

- 5       - comprise at least 0.005%wt of one or more nonionic surfactants;  
      - have pH  $\geq$  9;

10       and are further characterised in that the sole antimicrobially active component is the one or more nonionic surfactant(s) and in that the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is  
15       antimicrobially effective.

2. Antimicrobially effective aqueous solutions according to claim 1 characterised in that the nonionic surfactants are chosen from:

- 20       - ethoxylated aliphatic alcohols  $RO(C_2H_4O)_xH$  wherein R is an aliphatic group containing 6-20 carbon atoms and x is 2-20;  
      - ethoxylated alkyl-phenols  $R-C_6H_5O(C_2H_4O)_xH$  wherein R is an aliphatic group containing 4-15 carbon atoms  
25       and x is 2-20;  
      - amine oxides  $RRRN^+O^-$  wherein one of R is an aliphatic group containing 6-20 carbon atoms and the other Rs are aliphatic groups containing 1-3 carbon atoms.

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3. Antimicrobially effective aqueous solutions according to claim 2 characterised in that the nonionic surfactants have an HLB of 9-14.

4. Antimicrobially effective aqueous solutions according to claims 2 or 3 characterised in that the nonionic surfactants are chosen from ethoxylated alcohols and ethoxylated alkylphenols.

5. Antimicrobially effective aqueous solutions according to claims 1-4 characterised in that the pH is between 9.5 and 12.5.

10 6. Antimicrobially effective aqueous solutions according to claims 1-5 characterised in that the concentration of nonionic surfactant is at least 0.02%wt.

15 7. A process for antimicrobial treatment of animate and inanimate surfaces characterised in that an aqueous solution which comprises at least 0.005%wt of one or more nonionic surfactants, which has  $\text{pH} \geq 9$ , in which the sole antimicrobially active component is the one or more nonionic surfactant(s) and in which the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is antimicrobially effective, is applied to such surface.

25 8. The use of an aqueous solution which comprises at least 0.005%wt of one or more nonionic surfactants, which has  $\text{pH} \geq 9$ , in which the sole antimicrobially active component is the one or more nonionic surfactant(s) and in which the aqueous solutions do not contain another known antimicrobially active component in a concentration at or above the concentration at which it is antimicrobially effective, for antimicrobial treatment of animate and inanimate surfaces.

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# INTERNATIONAL SEARCH REPORT

International Application No

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C11D3/48 C11D1/66 A01N31/02 A01N31/08 A01N33/24

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C11D A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, CHEM ABS Data

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A		5
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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# INTERNATIONAL SEARCH REPORT

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PCT/EP 00/11711

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Information on patent family members

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THE (1970)